Natural Rubber Research, 21 (1&2): 139-144, 2008 SHORT SCIENTIFIC COMMUNICATION

P- PROTEIN IN THE SIEVE ELEMENTS OF *HEVEA BRASILIENSIS* TREES AFFECTED BY TAPPING PANEL DRYNESS

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Pramod, S., Thomas, V. and Rao, K.S. (2008). P-protein in the sieve elements of *Hevea brasiliensis* trees affected by tapping panel dryness. *Natural Rubber Research*, 21(1&2):139-144.

The structural features of P-proteins in the sieve elements of *Hevea brasiliensis* were studied in relation to Tapping Panel Dryness (TPD) and stimulation of affected bark with ethephon and vitex (a proprietary chemical formulation). The P-protein appears as a narrow, elongated structure in the sieve plate of healthy trees and unaffected parts of TPD affected trees whereas the TPD affected area of trees on rest for three years showed thick P-protein plugs. When the affected bark of TPD trees on rest were treated with ethylene the P-protein present in the active sieve element disappeared, while stimulation with vitex resulted in a positional change in P-protein. Callose deposition that followed in such stimulated sieve elements led to their senescence. The sieve element plugging that regulate photosynthate translocation with reference to TPD is discussed.

Key words: *Hevea brasiliensis*, P-protein, Sieve element, Stimulation, Tapping Panel Dryness.

P- protein helps plants to tide over adverse situations by agglomerating adjacent to the sieve plates and regulating the movement of solutes (Esau *et al.*, 1966; Parthasarathy, 1975). P- protein appears at sites of injury as an immediate wound response which is followed by deposition of definitive callose, lignin and suberin to seal off the wounded cells (Lipetz, 1970; Fahn, 1982; Thomas *et al.*, 1995).

Natural rubber latex is harvested from *Hevea brasiliensis*, by controlled wounding (tapping) of the articulately branched latex vessels in the bark of the tree trunk. The injury to the bark leads to a series of anatomical and histochemical changes in cells adjacent to the wound. A thin shaving of the bark is removed by tapping, leaving the cambium innermost and part of the bark uninjured (Thomas *et al.*,1995). Bobilioff (1923) and Premakumari and Panikkar (1992) suggested that wound healings and bark regeneration in *H. brasiliensis* are related. The residual bark left uncut is instrumental in healing and tissue regeneration at the site of injury. On tapping, the tree initiates certain defense mechanisms to regulate the excess loss of photosynthates through latex. P- protein occur in the phloem tissues of young stems and bark of mature trees after wounding (Wu and Hao, 1987; 1990). The sieve elements (Phloem) adjacent to wounds are blocked by definitive callose and the area of active sieve elements is limited to

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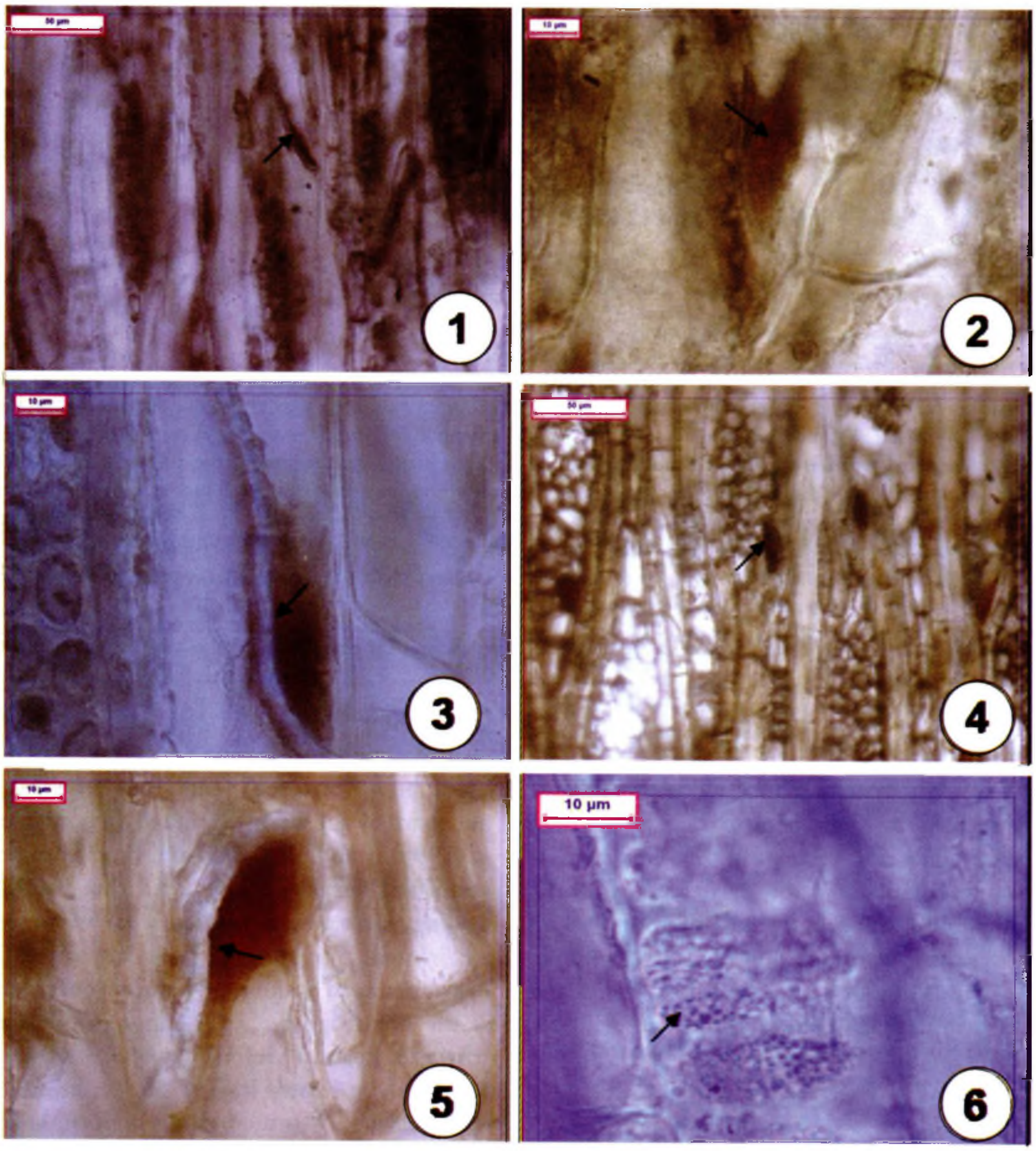

Fig. 1. The healthy tapped tree showing narrow elongated P-protein plug (arrow) on sieve plate area (TLS)
Fig. 2. The enlarged view of P-protein plug (arrow) on sieve plate of healthy bark (TLS).
Fig. 3. The unaffected area of the TPD affected tree showing narrow strand of P-protein (arrow) near to sieve plate
Fig. 4. The TPD affected trees on rest showing much larger P-protein (at arrow) plug on the sieve plate (TLS)
Fig. 5. The enlarged view of large P-protein plug (at arrow) on the sieve plate of TPD affected area
Fig. 6. The TPD affected area on rest showing penetration of P-protein into the sieve pores (at arrow) (TLS)

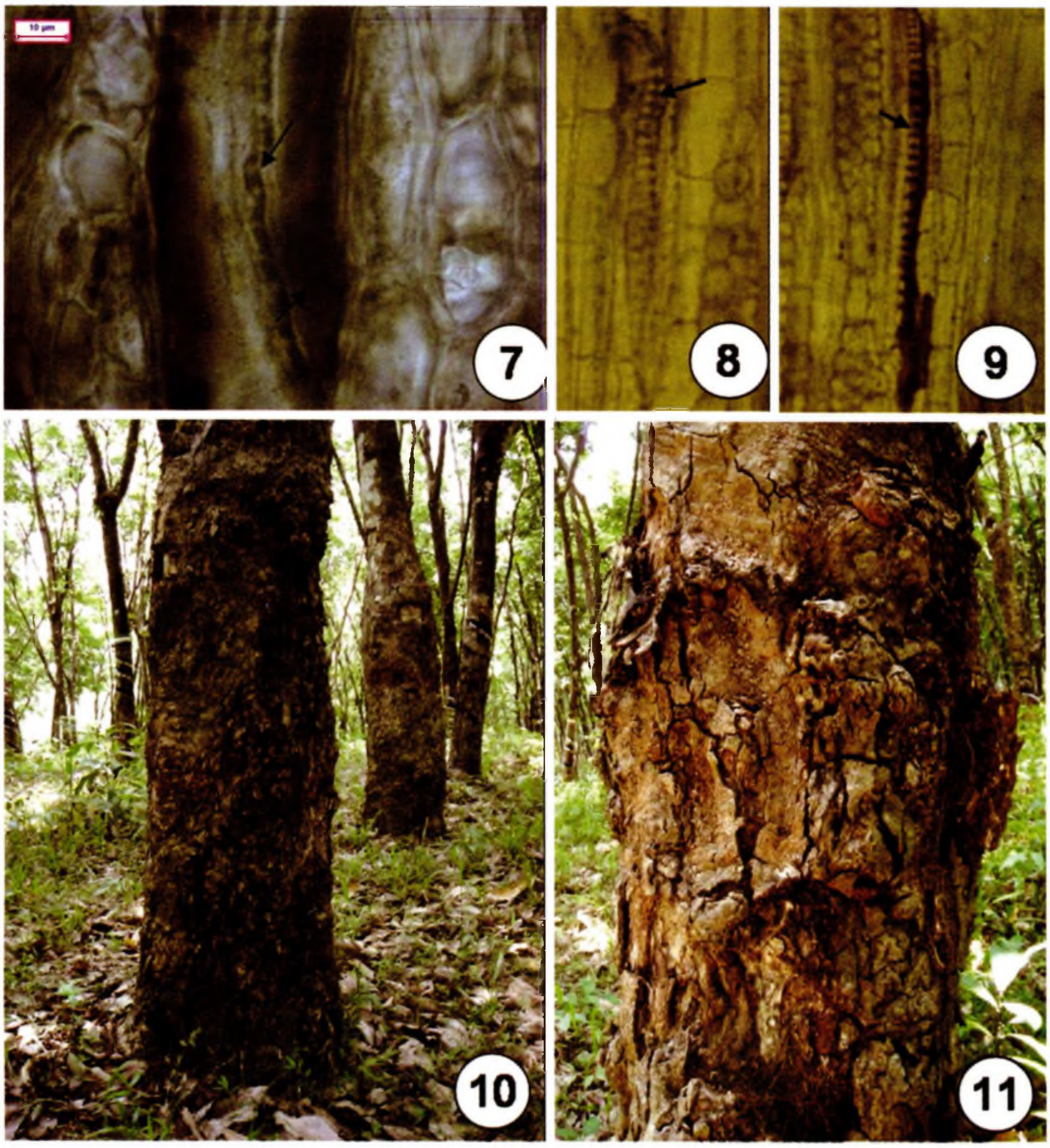

Fig. 7. The affected area stimulated with vitex showing P-protein plug (arrow) near sieve plate (TLS)
Fig. 8, 9. An initial stage of lignification (arrow) of sieve plate covered by definitive callose
Fig. 10, 11. TPD affected region of tree showing bulging and cracking of bark

the inner bark adjacent to the cambium. In bark tissues, sieve elements form the main cells involved in the translocation of photosynthates to the site of utility and storage.

Tapping panel dryness (TPD) of *H. brasiliensis* is a syndrome resulting in the cessation of latex flow from the tapping cut. Incidence of TPD is related to over-exploitation of bark.

Stimulation with ethephon, a practice in rubber plantations to get higher yield may cause upto 20 per cent incidence of TPD (Jacob and Krishnakumar, 2006). One of the important symptoms of TPD is the depletion in translocation of photosynthates by reducing the area of active sieve elements (Pramod, 2007). Since P-protein is an important component of the active sieve elements, the present investigation explores the structural features of P- protein in healthy and TPD affected trees of *H. brasiliensis.*

The experiment was conducted on 21 year old trees of clone RRII 105 planted in the Rubber Research Institute of India Farm at Kottayam, Kerala. Four sets each of six trees were selected to represent healthy trees, trees affected by TPD and rested for three years, trees applied with ethephon as a band of 5 cm on the affected bark and trees applied similarly with 'Vitex' a proprietary chemical produced by MRP Agrochemicals and marketed by Barath Agrotech, Pala, Kerala, India as a remedy for TPD. Bark samples were colleted after three months of application of the chemicals from the tree trunk immediately next the tapping cut in healthy trees and affected and non-affected area of TPD trees with the help of a chisel and hammer. Radial longitudinal sections (RLS) and Tangential longitudinal sections (TLS) of 40 µm thickness were cut and stained with mercuric chloride-bromophenol blue (Mazia *et al.*, 1953). The proteinaceous nature of the substance found in the sieve elements was confirmed by staining sections with Acid fuschin (McCully, 1966).

Bark samples collected immediately next to the tapping cut on healthy trees showed the presence of P-protein as a narrow elongated structure in the sieve elements near to the sieve plate (Figs.1 and 2). In the unaffected parts of the TPD affected trees, P-protein plug appeared adjacent to the sieve plate as a narrow elongated structure (Fig.3). In most cases, the entire sieve plate was not covered by P-protein. The bark of TPD affected trees on rest for a period of more than three years, thick P-protein plugging was localized in most of the functionally active sieve element area in the soft bark (Figs. 4 and 5). The P-protein penetrating through the sieve pore was also evident (Fig. 6). The P-protein plug appeared as a large mass which covered most of the sieve plate. Such large mass of P-protein was mostly found in the sieve plate adjacent to the ray cell.

When ethephon was applied at fortnightly intervals for three months to the affected bark of the TPD trees on rest, the P-protein in the active sieve elements disappeared. P-protein plugging was a rare event in the ethephon treated affected areas where most of the sieve plates were covered by thick deposition of definitive callose.

On the vitex applied trees latex production continued for about four months after which there was complete stoppage of latex flow. Bark samples collected at this stage showed certain structural and positional changes in P-protein (Fig.7). The P-protein plug appeared a little way from the sieve plate on the opposite side of the plate. They were broad and elongated and covered almost complete length of the sieve plate.

The formation of P-protein and wound healing materials like lignin and suberin, may be preventing the excessive loss of solute and cell organelles from the wounded tissue and consequently mortality of tissue (Cronshaw, 1981; Wu and Hao, 1990; Thomas *et al.,* 1995; Thomas, 2003).

The first visible symptom of TPD is late dripping of latex resulting in excessive loss of cell organelles and photosynthates rendering the bark unproductive (Gomez and Moir, 1979). The latex vessels get their nourishments from the surrounding sieve elements and parenchyma cells (Edgar, 1958). The penetration of P-protein plug into the sieve pore indicates a regulation of translocation in the sieve element. The thick deposition of P-protein in the sieve elements adjacent to the cambial zone in the affected trees on rest may be due to the stress due to over exploitation developed.

Initiation of TPD could be due to the excessive level of ethylene produced in the tissue as a result of intensive tapping or due to the exogenous application of ethephon (Pavanjothy *et al.*, 1979; Vijayakumar *et al.*, 1990). Ethephon stimulation makes the bark productive for a short period. Following to stimulation the P- protein may disappear and the sieve plates are deposited with thick callose. After the formation of the callose, these sieve elements underwent lignification thereby completing the senescence process (Figs.8 and 9) as observation were reported by Evert (1977) for *Tetragonia* and *Lycopersicum.* The amount of callose observed surrounding sieve plate pores varies according to the degree of injury to the sieve elements (Cronshaw, 1981).

Many functions have been attributed to P-protein which include mobilization of solute in the functionally active sieve elements and wound stress responses. The P-protein formation after each tapping in *H. brasiliensis* could be a temporary wound response since the latex is being collected by severing the adjacent loci in the tapping panel at short intervals for several years. The formation of P-protein has been reported in developing and wounded sieve elements in *H. brasiliensis* by Wu and Hao (1990). The lipoid materials were found to be more in the TPD affected tissues (Pramod, 2007) which may also result in increased P-protein plugging. The P-protein may appear in different structural forms with respect to ontogeny of sieve elements and degree of stress to the plant. Structural difference of the P-protein plug in the TPD affected and unaffected area may be due to the differences in the metabolic activity of the element in relation to the time at which they undergo maturation.

In TPD affected regions of trees (Figs. 10 and 11) the altered cambial activity may result in the rapid differentiation of cambial derivatives (Thomas *et al.,* 2006). The metabolic conditions of a rapidly differentiating cell may differ from its original rhythmic state. The P-protein appearance is directly linked to the early stages of sieve element differentiation (Cronshaw, 1975). Following TPD, the downward translocation through the sieve element of inner bark is regulated by the deposition of P-protein.

The authors are thankful to Dr. N.M. Mathew, former Director for giving the permission to carry out the work at RRII. Thanks are also due to Dr. James Jacob, Director and Dr. Y. Annamma Varghese, Joint Director (Crop Improvement), RRII for encouragement.

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